

SURVEY OF VIRUSES AFFECTING FABA BEAN IN SIX ARAB COUNTRIES

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Abstract

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A field survey of faba bean (*Vicia faba* L.) for viruses in six Arab countries showed the presence of nine viruses. Bean leaf roll virus (BLRV), bean yellow mosaic virus (BYMV), broad bean mottle virus (BBMV) and to a lesser extent broad bean stain virus (BBSV) were the most common. When testing with ELISA 789 samples with symptoms suggestive of virus infection collected from Egypt, Lebanon, Morocco, Sudan, Syria and Tunisia, BBMV was detected in 203 samples, BBSV in 151, broad bean true mosaic virus (BBTMV) in 7, broad bean wilt virus (BBWV) in 47, BYMV in 314, cucumber mosaic virus (CMV) in 96, pea enation

mosaic virus (PEMV) in 31, and pea seed-borne mosaic virus (PSbMV) in 49 samples. Identity of selected field isolates was confirmed by electron microscopy and host reaction studies. In a yield experiment, infection with BYMV, BBMV and BBSV 11 weeks after sowing (pre-flowering) led to 81, 54 and 84% yield loss, respectively. Inoculation with the same viruses 15 weeks after sowing (flowering) and 20 weeks after sowing (pod setting) led to 56, 84 and 18%, and 39, 37 and 18% yield loss, respectively.

Key words: faba bean, viruses, Arab countries.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important food crop in many Arab countries. It is considered the main protein source for a large part of the population. Productivity of the crop is affected by a number of factors including viruses. Some 44 viruses are known world-wide to affect faba bean (3, 5, 9, 23), but only few of them were reported so far from Arab countries (1, 2, 10, 11, 16, 17). Tentative studies indicated the presence of nine faba bean viruses in six West Asian and North African countries, and the present study was undertaken to evaluate their incidence and the potential yield losses caused by the major ones. Results of more detailed studies on broad bean stain virus (BBSV) and broad bean mottle virus (BBMV) have already been published or are ready for publication (18, 19).

MATERIALS AND METHODS

Field observations and sample collection. Faba bean fields were visited during March-April 1985, 1986 and 1987 in Syria, Tunisia and Morocco. In Lebanon such visits were made in April of 1985, and in Egypt and Sudan in January 1986. Samples of faba bean with symptoms suggestive of virus infection were collected from farmers' fields and from experimental plots of agricultural research stations. Samples were brought to the laboratory in Aleppo and each sample was split into two portions. One was desiccated over calcium

chloride for virus recovery and electron microscopy when needed, and the other was extracted in 0.2 M phosphate buffer, PH 6.0, and used for ELISA.

Serological tests. Antisera to broad bean mottle virus (BBMV), broad bean stain virus (BBSV), cucumber mosaic virus (CMV) and pea enation mosaic virus (PEMV) had been produced in our laboratory. Antisera to bean leaf roll virus (BLRV = pea leaf roll virus) and subterranean clover red leaf virus (SCLRV) were provided by J.W. Ashby (DSIR, New Zealand), to bean yellow mosaic virus (BYMV) by J. Raddles (University of Adelaide, Australia), to broad bean true mosaic virus (BBTMV) by H. Rohloff (BBA, Braunschweig, FRG), and to broad bean wilt virus (BBWV) and pea seed-borne mosaic virus (PSbMV) by D.Z. Maat (IPO, Wageningen, the Netherlands).

The procedure for direct double-sandwich ELISA was as described by Clark and Adams (8), but for sample extraction 0.2 M phosphate buffer, PH 6.0, was used.

Electron microscopy. Selected field samples were examined with the electron microscope (EM) for virus particles to confirm identity revealed by serology. Leaf samples, either fresh or desiccated over calcium chloride, were then chopped in sodium phosphotungstate (PTA 2% PH 6.5) for negative staining before viewing with the EM.

Yield loss assessment. A field experiment was conducted

during the growing season 1986 – 1987 in Tel Hadya, Syria to assess potential losses induced by three viruses separately, viz. BBMV, BBSV and BYMV, and by a mixed infection of BBMV and BYMV. The following virus isolates from Syria were used: SV48 – 85 for BBMV, SV173 – 85 for BBSV, and SV205 – 86 for BYMV. Each virus or virus mixture was mechanically inoculated to 80 faba bean plants grown in a 1.8 × 4m plots and replicated four times in a randomized complete block design. Three inoculation dates were evaluated, viz. just before flowering (around 11 weeks after sowing), during flowering (15 weeks after sowing), and during pod setting (20 weeks after sowing). Plots were sprayed with insecticides weekly to minimize vector activity. At maturity, plots were harvested, dry seeds were collected and yield data were statistically analyzed.

RESULTS

Field surveys. In all countries surveyed by sample testing and field observation for symptoms, BLRV was the most common virus on faba bean. Symptoms observed were yellowing and leafrolling of the upper leaves (Fig. 1B), and when young plants were infected all leaves showed yellowing and leafroll symptoms (Fig. 1A). Affected leaves were thick and leathery. BLRV – infected plants produced little or no pods and were usually more severely infected by *Botrytis* spp. than healthy ones. Incidence of infection in faba bean fields ranged from 0.5 to 20%, but in exceptional cases up to 100% leading to total crop loss, such as in the coastal area of Syria during the early spring of 1986. Identification of BLRV was confirmed by serology when a Syrian field isolate with typical BLRV symptoms was tested with antisera to BLRV and SCRLV (Table 1). Reaction was stronger with BLRV antiserum than with SCRLV antiserum.

Symptoms of mechanically transmissible, mosaic-including viruses were less diagnostic than those of BLRV, often the result of complex infections. Many samples with symptoms suggestive of virus infection collected during field surveys were therefore tested serologically. Selected field samples were also tested by electron microscopy and inoculation onto indicator plants.

Table 1: ELISA values (A405) obtained when leaf extracts from faba bean plants with leaf roll symptoms were tested with BLRV and SCRLV antisera. Plates were coated with 10 µg/ml of gammaglobulins and the conjugate dilution used was 1:800.

Test sample	Substrate incubation time	Antiserum	
		BLRV	SCRLV
Leafroll – diseased faba bean (Aleppo)	2 hours	0.30	0.13
	overnight (4°C)	1.13	0.42
Healthy faba bean	2 hours	0.04	0.02
	overnight (4°C)	0.15	0.07

Many of the 789 samples tested by ELISA with eight different antisera reacted to one or more antisera (Table 2). BYMV was the most common mechanically transmitted virus present. Relative incidence of BYMV was high in Egypt (in 47 out of 70 samples tested: 47/70), Sudan (128/254) and Syria (90/269). BBMV was detected mostly in Tunisia (80/137), followed by Syria (72/269) then Sudan (46/254). In Tunisia BBMV was more common than BYMV, and it was the only country surveyed where BBMV incidence was higher than that of BYMV.

Mixed infections were as common as single ones (Table 2). Around 31% (247/789) of the samples did not react to any of the eight antisera used. This suggests the presence of viruses other than those reported in this study.

Electron microscopy. Negative staining of selected isolates, initially identified by serology, revealed virus particles which confirmed their serological behavior. Faba bean plants with the isolates SuV98 – 86, SV205 – 85, SV231 – 85 contained 700 – 800 nm long flexuous particles typical of BYMV (Fig. 2A). Preparations with isolates SV48 – 86 and TV75 – 85 showed abundant isometric particles ca 25 nm in diameter and typical of BBMV (Fig. 2B). Isolate EV319 – 86 was associated with isometric particles 25 nm in diameter typical of BBMV. Faba bean tissue infected with isolates SV173 – 85 and TV73 – 85 contained virus particles ca 25 nm in diameter typical of BBSV and those with isolate SV36 – 86 contained virus particles ca 28 nm in diameter, typical of CMV.

Symptomatology and host reactions of selected field isolates. BBMV produced pronounced systemic vein chlorosis soon developing into general chlorosis followed by some degree of recovery and thereafter a usually severe malforming mottling, marbling or mosaic and severe growth reduction in all cultivars tested (Fig. 3). The virus infected 11 legumes and 9 non-legume species out of 26 tested. Infection in several of them was symptomless, but major legume species, such as chickpea, lentil (Fig. 4) and especially pea, severely suffered from infection, the latter species usually with premature death (19).

All cultivars of chickpea, faba bean lentil, pea, vetches (*Vicia sativa* and *V. ervile*) and subterranean clover tested with BBSV were infected systemically. BBSV induced a mild mottle on faba bean (Fig. 1C), and seeds of naturally infected plants are often stained with localized brown necrosis around their periphery. It caused vein and leaf necrosis on pea often followed by plant death or systemic leaf mottling. All other infections were symptomless, as were those of 12 wild legume species tested. Two additional wild legumes, *Medicago arabica* and *Trifolium spumosum* had obvious mosaic symptoms (18).



Figure 1. Symptoms for some viruses in faba bean: A and B bean leafroll virus; A) after early and B) after late infection in the field; C) broad bean stain virus after inoculation in the greenhouse; D) pea enation mosaic virus after natural infection. (A and B photographs L. Bos; C) and D) photographs IPO, Wageningen.)

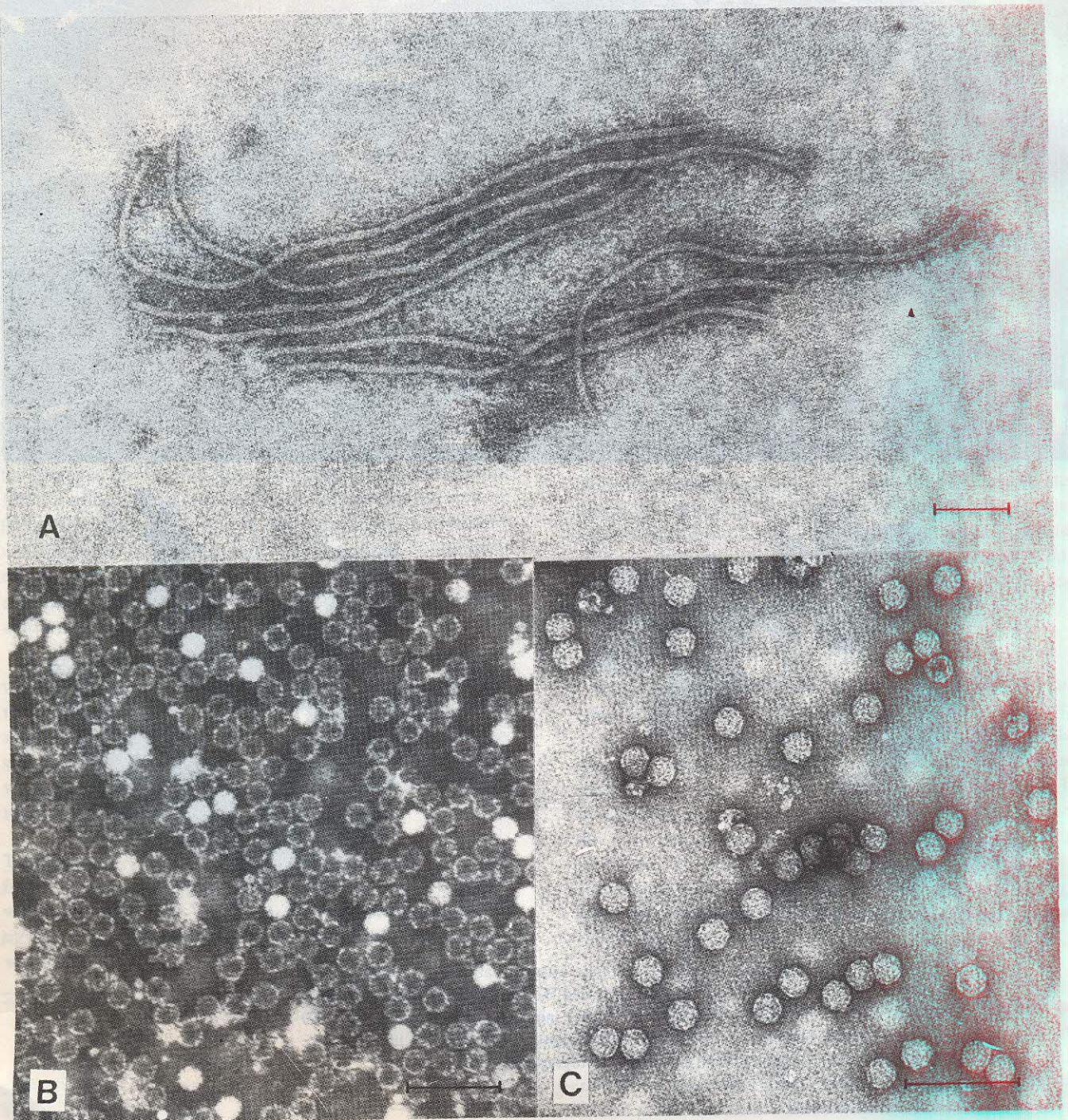


Figure 2. Particles of some viruses of faba bean seen with the electron microscope: A) bean yellow mosaic virus; B) broad bean mottle virus, usually occurring in plant sap in high concentrations; C) bean leafroll virus, in plants occurring in phloem tissue only. A and B) crude sap preparations, C) purified preparation. (Photographs IPO, Wageningen). Magnification bars represent 100 nm.

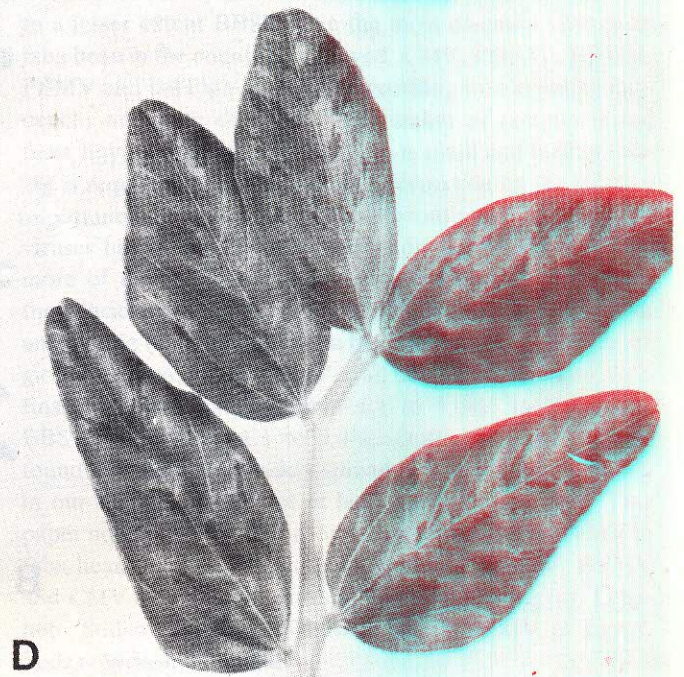
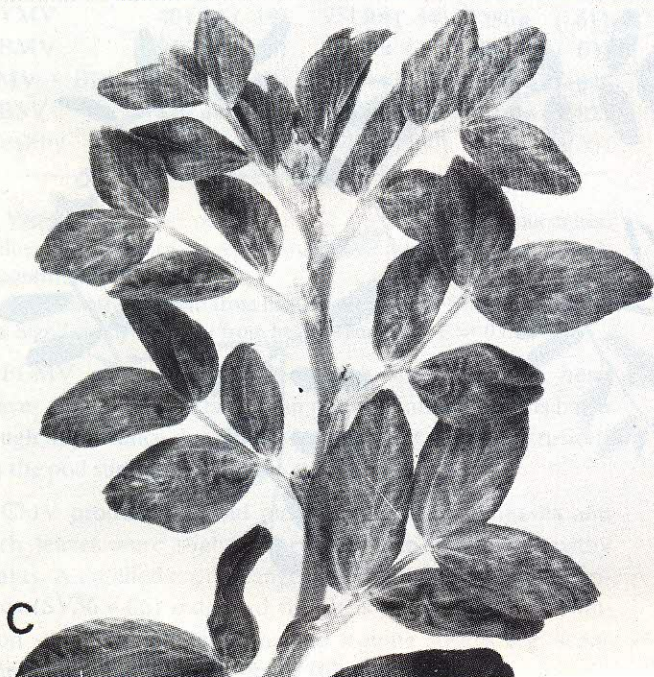
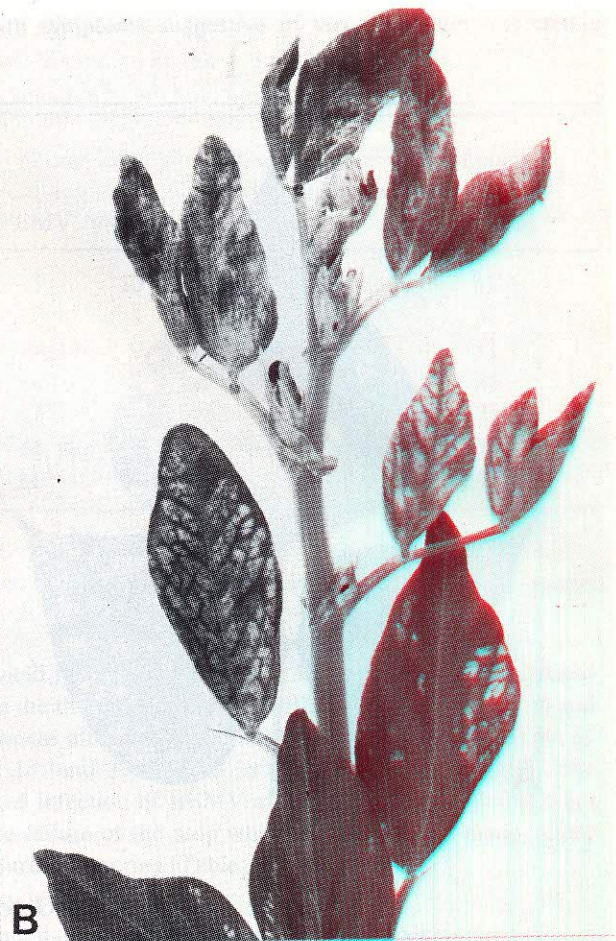
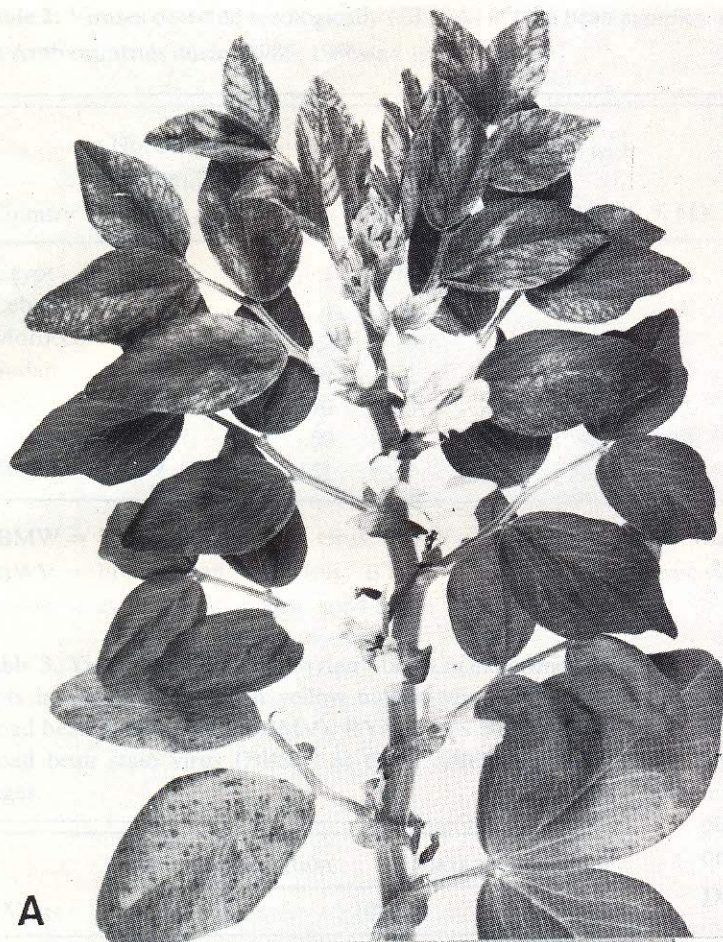


Figure 3. Symptoms in faba bean of broad bean mottle virus (isolate from Tunisia in «Compacta» and ICARDA breeding line, respectively) (A and B), and of bean yellow mosaic virus (Isolate from Egypt) in «Compacta» (C and D); all after inoculation in the glass-house. (Photographs IPO, Wageningen.)

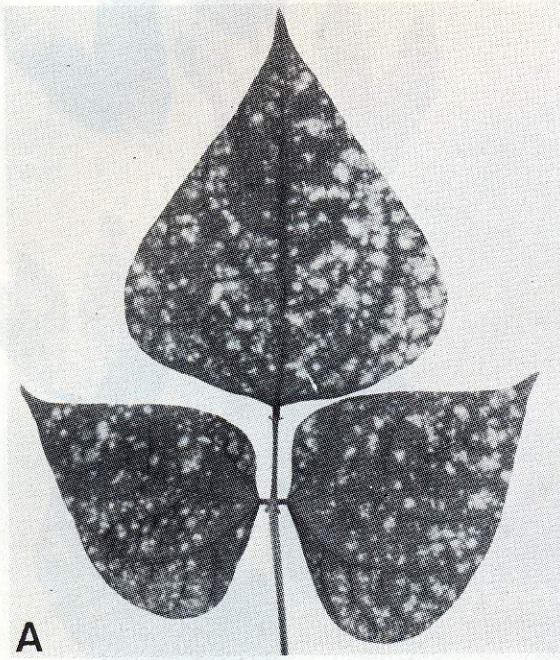


Figure 4. Symptoms of broad bean mottle virus in other legumes, both after inoculation in the glasshouse: A) in *Phaseolus vulgaris* «Double White», and B) in Lentil (*Lens esculenta*) «Syrian Local Small». (Photographs IPO, Wageningen).

Table 2. Viruses detected serologically (ELISA) in faba bean samples with symptoms suggestive of virus infection collected in six Arab countries during 1985, 1986 and 1987.

Country	No. of plants tested	No. of plants infected with								No. of plants with single infection	No. of plants where virus was not identified
		BBMV	BBSV	BBTMV	BBWV	BYMV	CMV	PEMV	PSbMV		
Egypt	70	4	4	0	4	47	10	7	10	39	12
Lebanon	44	0	6	0	0	5	0	0	1	12	32
Morocco	15	1	2	0	0	1	0	0	0	4	11
Sudan	254	46	19	1	0	128	6	4	3	119	85
Syria	269	72	70	3	20	90	35	10	20	93	73
Tunisia	138	80	50	3	23	43	45	10	15	36	34
Total	789	203	151	7	47	314	96	31	49	303	247

BBMV = broad bean mottle virus; BBSV = broad bean stain virus; BBTMV = broad bean true mosaic virus; BBWV = broad bean wilt virus; BYMV = bean yellow mosaic virus; CMV = cucumber mosaic virus; PEMV = pea enation mosaic virus; PSbMV = pea seed-borne mosaic virus

Table 3. Yield^a of faba bean «Syrian Local» in experimental plots inoculated with bean yellow mosaic virus (BYMV), broad bean mottle virus (BBMV), BYMV plus BBMV, and broad bean stain virus (BBSV) at three different growth stages.

Virus	Time of inoculation (days after sowing)		
	76	107	140
BYMV	404 ** (19)	931 ** (44)	1286* (61)
BBMV	955 ** (46)	335 ** (16)	1311 ** (63)
BYMV + BBMV	70 ** (3)	186 ** (9)	1339 ** (64)
BBSV	339 ** (16)	1727 * (82)	1713 * (82)
Healthy	2095 (100)	2095 (100)	2095 (100)

a) Yield in grams per plot of 1.8 × 4 meter replicated four times. Values between brackets represent % yield as compared to the healthy control.

* Significantly different from healthy control at P = 0.05

** Significantly different from healthy control at P = 0.01

PEMV caused chlorotic - whitish spots on faba bean leaves (Fig. 1D), and faba bean pods on infected plants had a rough appearance due to the formation of small protrusions on the pod surface.

CMV produced a mild mosaic on faba bean leaves and such leaves were slightly narrower than those of healthy plants. A detailed host - range study on one of the CMV isolates (SV36 - 86) indicated that it is more related to common strains of the virus than to legume strains e.g. seed-transmitted in *Phaseolus* beans (6).

BBMV infection of faba bean was mild as indicated by the light vein chlorosis and leaf narrowing observed. None of the faba bean plants inoculated with this virus showed wilting symptoms.

Yield loss evaluation. All viruses tested caused significant yield losses. Early inoculation resulted in higher percentages

of yield reduction. When faba bean plants were inoculated with the three viruses BYMV, BBMV, and BBSV 11, 15 and 20 weeks after sowing induced around 81, 54 and 84; 56, 84 and 18; and 39, 37 and 18% yield loss, respectively. The mixed infection of BBMV and BYMV caused almost complete failure of the crop when inoculation was made before or during flowering (Table 3).

DISCUSSION

The field survey showed that BLRV, BYMV, BBMV and to a lesser extent BBSV were the most common viruses on faba bean in the countries surveyed. CMV, PSbMV, BBWV, PEMV and BBTMV, arranged according to decreasing incidence, were also detected. The number of samples tested from Egypt, Lebanon and Morocco is small and further testing is required for more reliable information on the relative importance of the viruses in these countries. Some of these viruses have been reported earlier on faba bean in one or more of the six countries with little or no information on their incidence (1, 10, 11, 16, 17, 18, 19, 20, 24). Bourbah and Fezzaz (7), surveying faba bean fields in the Meknes region, Morocco, found BBMV in all the fields examined, with final incidences ranging from 1.1 to 5.0%; incidences of BBSV varied from 1.1 to 2.4%. Ouffroukh (22) recently found that BBMV is widely spread in Algeria, not included in our survey, though not of high incidence (1 - 3%). Our paper now is the first report on the occurrence of BBTMV in faba bean in Sudan, Syria and Tunisia; of BBWV, BYMV and CMV in Syria and Tunisia; of PSbMV in Egypt, Lebanon, Sudan, Syria and Tunisia; and of PEMV in Egypt, Sudan, Syria and Tunisia.

BLRV which is transmitted by aphids in a persistent manner, may be widespread throughout West Asia and North Africa and is often prevalent, as in the coastal region of Syria. The virus has earlier been reported to be prevalent in Iran on faba bean and other food legumes (14, 15). It greatly increases susceptibility to *Botrytis fabae* and *B. cinerea* (this publication and Omar *et al.* (21)), which greatly adds to the

importance of the virus.

The yield experiment showed a significant reduction in faba bean yield in response to infection with BBMV, BBSV, BYMV and mixed infection of BBMV + BYMV. Yield loss reported here were similar to those reported earlier for BYMV (13, 15) and for BBMV and BBSV (7, 22). Highest losses by BBSV and BYMV occurred when plants were inoculated early in the season before flowering. With these viruses yield loss is more or less proportional to duration on infection as also found for other viruses (4). With BBMV losses were highest when inoculation was during flowering. Highest sensitivity to infection at a certain critical stage of plant development has been reported earlier for a virus affecting peas (12). At early inoculation the effect of BBMV and BYMV when occurring together was more than cumulative.

Even though yield losses due to infection at pre-flowering or flowering growth stages are high in individual plants, field incidence of infection with the three viruses usually is not high early in the growing season due to low vector activity at that time. However, high potential field losses of 39, 38 and 19% for BYMV, BBMV, and BBSV, respectively, were still obtained at late infection and these figures may represent actual losses in some countries. Incidences of BYMV infection up to 100%, in addition to some BBMV infection, late in the growing season (January) are not uncommon in the

Sudan. High BBSV incidences in Syria and Tunisia are likely when beetle activity is high. In many locations in Syria beetles are active in March and April. With a few early foci of the seed-borne BBSV within the crop infection, the virus may spread rapidly. Mild symptoms of BBSV, or their absence, when plants are infected late (during pod setting), may lead to under estimation of actual losses. Random surveying of faba bean fields for BBSV, therefore, is needed to provide a better understanding of the importance of this virus.

The surveys reported here have so far not been made systematically, covering the entire faba bean growing areas of the regions concerned. The data presented nevertheless show a high incidence and wide distribution of some viruses well-known from other parts of the world (BLRV and BYMV), but more so of other viruses earlier though to be of mere academic interest (BBSV and especially BBMV). The wide host ranges, including perennial legumes, of most of the viruses found in faba bean and seed transmission of several of them (BBMV, BBSV, BBTMV, BYMV and PSbMV) further add to the importance of these viruses in crop improvement programs in West Asia and North Africa (5).

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الملخص

مكوك خالد ، لوت بوس ، عصمت عزام ، صفاء القمري واليس رزق الله . 1988 . مسح للفيروسات التي تصيب الفول في ستة بلدان عربية . مجلة وقاية النبات العربية 61:6 - 53 .

المنقول بوساطة البذور «PSbMV» في 49 عينة . كما تم التأكد من هوية هذه الفيروسات بدراسة عينات حقلية مختارة منها بوساطة المجهر الإلكتروني ، وبدراسة تفاعلها مع مجموعة كبيرة من النباتات الدالة . وفي تجربة لتقدير تأثير هذه الفيروسات على الغلة ، تبين أن إصابة النبات في مرحلة ما قبل الأزهار - 11 اسبوعاً بعد الزراعة - بفيروسات موزاييك الفاصولياء الأصفر وتبرقش الفول وتلون بذور الفول ، أدت إلى خسارة في المحصول قدرت بـ 80% ، 55% و 84% على التوالي . كما أدى إلحاق النباتات بالفيروسات الثلاث نفسها والذي قمنا بإجرائه في مرحلة الأزهار - بعد 15 اسبوعاً من الزراعة - وفي مرحلة تكون القرون - بعد 20 اسبوعاً من الزراعة - إلى خسارة في المحصول قدرت بـ 55% ، 84% ، 18% على التوالي في الحالة الأولى ، و 39% ، 38% ، و 19% على التوالي في الحالة الثانية .

كلمات مفتاحية: فول ، فيروسات ، بلدان عربية .

أظهر مسح حقل للفيروسات التي تصيب الفول في ستة بلدان عربية (مصر ، لبنان ، المغرب ، السودان ، سورية ، تونس) وجود تسعة فيروسات تصيب هذا المحصول . وكان أكثر هذه الفيروسات انتشاراً هو فيروس التفاف أوراق الفول «BLRV» ، تلاه فيروس موزاييك الفاصولياء الأصفر «BYMV» ، ثم فيروس تبرقش الفول «BBMV» ، وبدرجة أقل فيروس تلون بذور الفول «BBSV» .

عند إجراء اختبار «الليزا» على 789 عينة أظهرت أعراضاً توحي بإصابة فيروسية - كان قد تم جمعها خلال الفترة 1985 - 1987 من البلدان العربية آفة الذكر - تم كشف فيروس تبرقش الفول في 203 عينة وفيروس تلون بذور الفول في 151 عينة ، وفيروس موزاييك الفول الحقيقي «BBTMV» في سبع عينات ، وفيروس ذبول الفول «BBWV» في 47 عينة ، وفيروس موزاييك الفاصولياء الأصفر في 314 عينة ، وفيروس موزاييك الخيار «CMV» في 96 عينة ، وفيروس الموزاييك والزوائد في البازلاء «PEMV» في 31 عينة ، وفيروس موزاييك البازلاء

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